REMARKS

The Invention

Applicants have discovered that it is possible to create artificial P-selectin ligands by combining amino acid sequences containing tyrosine sulfation sites with sialyl Le^x addition sites. These sites may originate in different polypeptides and be inserted into a third, carrier polypeptide, or the sites may originate from the same polypeptide and be repositioned relative to one another. Generally, the claimed invention features purified nucleic acids encoding artificial P-selectin ligand polypeptides that contain a tyrosine sulfation site and a sialyl Le^x addition site, wherein at least one of the sites is located at an amino acid position where it does not naturally occur. The invention also features vectors and cells containing the claimed nucleic acids.

The Office Action

Claims 10, 12-14, 24, and 25 are pending in this application. All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for a lack of enablement and insufficient written description. All pending claims are further rejected for indefiniteness under 35 U.S.C. § 112, second paragraph.

Support for Amendments

Support for the amendment to claim 10 is found in the specification at page 25, line 19, through page 26, line 17. No new matter is introduced by this amendment.

A "marked up" version of the claim showing the changes made and an appendix of the claims as pending are attached.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 10, 12-14, 24, and 25 stand rejected, under 35 U.S.C. § 112, first paragraph, based on both lack of enablement and an inadequate written description. Each of these rejections is addressed below.

Enablement

Claims 10, 12-14, 24, and 25 are rejected under 35 U.S.C. § 112, first paragraph, based on lack of enablement. Specifically, the Office states that the specification does not provide sufficient guidance for a skilled artisan to practice the claimed invention without undue experimentation because of the unpredictable nature of the art, and the disclosure of only a single working example. The Office further asserts that the exemplary P-selectin ligand disclosed in the specification works only in an *in vitro* binding assay while the invention has only *in vivo* therapeutic and diagnostic uses. This rejection is respectfully traversed.

The standard for enablement is articulated in *In re Wands* 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988). In defining the boundaries of undue experimentation, the *Wands* court stated that "the key word is 'undue' not 'experimentation'" and that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine." *Id.* at 737.

In assessing enablement in the present case, and in putting it in the context of the issues of predictability and the *Wands* factors, it is important to first remember that it was prior to Applicants' discovery that scientists believed that ligand conformation and the distance between the tyrosine sulfation and the N-linked sialyl Le^x sites in P-selectin ligands was intolerant to change, as evidenced by publications such as Larsen et al. (U.S. Patent No. 5,843,707; art of record). Larsen suggested the creation of no fewer than 10 different synthetic P-selectin ligands (column 9, line 36, through column 11, line 66). A common feature of all of the Larsen ligands was the presence of at least one tyrosine

sulfation site (residues 46, 48, and 51) linked to at least one N-linked glycosylation site (residues 65, 111, and 292) by the native amino acid sequence. Larsen was careful to generate artificial P-selectin ligands that only contained modifications outside of — what was believed to be — the critical recognition region. Implicit in this teaching is the belief that the three dimensional relationship between these sites must be maintained in order to ensure functionality.

Applicants, however, demonstrated that the structural requirements for creating functional P-selectin ligands are not so restrictive. In particular, Applicants demonstrated that the spatial relationship between the tyrosine sulfation site and the sialyl Le^x site is far more flexible and amenable to change than was previously thought. Specifically, the protein encoded by the nucleic acid described at page 24, lines 1-16, contains a Factor VIII tyrosine sulfation site fused upstream from a CD43 fragment that contains sialyl Le^x addition sites. The intervening sequence in this molecule is not the same as the native P-selectin ligand protein. Therefore, Applicants demonstrated that the prevailing belief as to the strict structural requirements required for an active P-selectin ligand was incorrect.

In light of Applicants' discovery, it is irrelevant to the issue of enablement what scientists once believed about the "fixed" nature of P-selectin ligands (Office Action, page 3). What is relevant is Applicants' demonstration that artificial P-selectin ligands can be produced which do not have fixed distance constraints between the sialyl-Le^x and tyrosine sulfation sites. In view of this discovery, Applicants submit that it is, in fact, quite predictable that other P-selectin ligands can be produced using the information provided in the specification, in combination with only routine experimentation.

Indeed, Applicants point out that experimentation in the production of artificial P-selectin ligands can occur at only three stages: (1) nucleic acid and protein design; (2) nucleic acid expression; and (3) protein testing to identify useful P-selectin ligands. As discussed in more detail below, the specification, combined with the relevant art, provides a clear strategy for designing P-selectin ligand nucleic acids and also provides

methods for expressing and testing the resulting candidate synthetic ligands, so that, after merely routine experimentation, the artisan will have identified other synthetic P-selectin ligands of this invention.

Nucleic Acid and Protein Design

Specifically, to identify useful P-selectin ligands, nucleic acids must first be designed that include a sialyl-Le^x addition site and a tyrosine sulfation site. The Office argues that the current claims encompass an unlimited number of nucleic acids, which encode an unlimited number of polypeptides, because the claims have no limitation on either the tyrosine sulfation site or the sialyl Le^x addition site. This statement is incorrect in light of the relevant art.

In particular, tyrosine sulfation does not occur on any tyrosine residue, as the Office asserts; rather, tyrosine sulfation is limited by the features of its flanking sequence. Consensus tyrosine sulfation sites have been identified and characterized and have been known in the prior art since at least as far back as 1986, as evidenced by publications such as Hortin *et al.* (1986, *Characterization of Sites of Tyrosine Sulfation in Proteins and Criteria for Predicting their Occurrence*, *Biochem. Biophys. Res. Comm.*, 141:326-333) and Huttner, (1988, *Tyrosine Sulfation and the Secretory Pathway, Ann. Rev. Physiol.*, 50:363-376) (both citations are art of record). Indeed, as early as 1986, Hortin *et al.* disclosed (page 331, emphasis added):

> Based on the foregoing analysis of amino acid sequences surrounding sulfation sites, **five simple rules** were empirically derived to aid in predicting the location of sites of sulfation. Tyrosine residues that are likely sites of sulfation are identified by the following criteria:
> 1) There is an acidic residue at position -1 or -2,
> 2) There are at least 3 acidic amino acid residues within 5 residues (positions -5 to +5) of the tyrosine residue,
> 3) No more than 1 basic amino acid residue are within 5 residues of the tyrosine,
> 4) No more than 3 hydrophobic residues (Ile, Leu, Phe, and Val) are within

5 residues of the tyrosine,
5) No cysteine residues are within 15 residues of the tyrosine.

Thus, tyrosine sulfation sites represent very particular sites, and these sites have been well characterized for many years.

With respect to the other P-selectin ligand sites, the sialyl Le^x addition sites, Applicants point out that these sites as well represent very specific sequences, and these sequences were described with particularity in Applicants' specification. For example, at page 25, lines 19-27, the consensus site N X S/T for N-linked sialyl Le^x addition is identified, and on page 26, lines 2-10, the practitioner is further taught to insert artificial glycan addition sites, such as sialyl Le^x sites, in regions that are hydrophilic in nature, on the outside (hydrophilic face) of a candidate ligand molecule. The specification (page 26, lines 10-14) goes on to provide a strategy for creating artificial sialyl Le^x sites at desired protein positions by selecting locations which only differ from the consensus sequence by a single amino acid. Applicants state that, preferably, the sites are created by substituting amino acids of similar charge or polarity.

At the time of filing, therefore, determinants of tyrosine sulfation and sialyl Le^x addition were well known. The specification, in combination with the prior art, enables a practitioner to create or identify these sites in either a naturally occurring, or a non-naturally occurring position. The stringency of the consensus sequences for tyrosine sulfation and sialyl Le^x addition implicitly limits the scope of the claims; therefore, the claims do not encompass an unlimited number of nucleic acids as asserted by the Office.

Nucleic Acid Expression and Protein Testing

As the Office is not suggesting that a practitioner could not successfully express a candidate P-selectin nucleic acid, the remainder of this rejection focuses on the issue of assessing the functionality of the candidate ligands. On this issue, Applicants submit that the skill in the art of ligand binding assays is extremely high. Most practitioners in this

area of molecular biology hold Ph.D.'s and often have significant post-doctoral training.

In addition, the specification provides ample guidance for testing the effectiveness of candidate proteins as P-selectin ligands. Specifically, a cell adhesion assay and an HL-60 cell rolling assay are described at pages 13 and 15, respectively, either or both of which can be used to measure the functionality of a P-selectin ligand. Therefore, producing, expressing, and testing candidate proteins for P-selectin ligand activity is nothing more than routine for the skilled artisan.

Working Examples

The Office further argues that the disclosure of only a single working example which is encompassed by the claim limitations is not enabling, in light of the unpredictable nature of the art. In response to this assertion, Applicants first direct the Examiner to the discussion presented above at pages 3-4. As indicated, in light of Applicants' discovery, the production of artificial P-selectin ligands is no longer unpredictable; indeed, no more than routine experimentation is required to create and identify other synthetic ligands falling within the claims.

Moreover, the reliance by the Office on a requirement for working examples is not supported by the law. The courts have been very clear that not a single working example is required for a disclosure to be enabling. As stated in *In re Long*, 368 F.2d 892, 895, 54 C.C.P.A. 835, 838 (C.C.P.A. 1966), and affirmed in *In re Borkowski and Venrooy*, 422 F.2d 904, 164 U.S.P.Q. 642 (C.C.P.A. 1970), "the absence of a working example, denominated as such, does not compel the conclusion that a specification does not satisfy the requirements of 35 U.S.C. 112." The present specification provides a working example that falls within the scope of the claims, and others which illustrate the general principles of the invention. These examples, combined with the teachings of the specification and the art, provide ample guidance to enable the skilled artisan to practice the invention as claimed.

Finally, on this issue, the Office has also not carried its burden of demonstrating that the disclosure provided by Applicants in their specification could not be used to produce P-selectin ligands. Other than an assertion concerning Applicants' unexpected discovery that the three dimensional structure of P-selectin ligands could be altered, the Office provides no evidence or specific reasons for why, or in what way, Applicants' disclosure is deficient. As stated in the case of *In re Marzocchi* (439 F.2d 220, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971), the first paragraph of § 112 "requires nothing more than objective enablement" and in a case in which the Patent Office questions the enablement of a claim:

> ... it is incumbent upon the Patent Office ... to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement.

Marzocchi at 370.

In Vivo Utility

The Office further asserts that Applicants' *in vitro* binding assay does not provide enablement for *in vivo* therapeutic and diagnostic uses (Office Action, page 3, paragraph 4). As an initial matter, Applicants point out that diagnostic assays involving P-selectin ligands would typically be carried out *in vitro*, and not *in vivo*. In addition, Applicants respectfully submit that the Office has erred in the interpretation of the law in this area. The court in *In re Brana*, 51 F.3d 1560. 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995) reversed a U.S.P.T.O. decision that *in vitro* activity did not support therapeutic applications *in vivo*. Furthermore, the burden is on the Office to give reasons for a lack of correlation between an *in vitro* model and an *in vivo* process. MPEP § 2164.02. The appellants in *In re Brana* used *in vitro* data from two lymphocytic leukemia cell lines to support claims to *in vivo* uses of chemotherapeutic agents. Similarly, Applicants demonstrate *in vitro* efficacy of

synthetic P-selectin ligands using an HL-60 cell rolling assay (Figure 13 and page 15). HL-60 is a human leukemia cell line. The Office has failed to meet its burden for rejection by giving no reason or documentary support for why data from the HL-60 model is not an acceptable correlate for a ligand's efficacy in inhibiting inflammation-related processes *in vivo*. Accordingly, this basis for the rejection is improper and should be withdrawn.

Written Description

Claims 10, 12-14, 24, and 25 also stand rejected under 35 U.S.C. § 112, first paragraph on the basis that the specification fails to provide a written description sufficient to support Applicants' genus claim to artificial P-selectin ligands. Specifically, the Office states that the specification provides no guidance on what might constitute a sialyl Le^x addition site or a tyrosine sulfation site, leading to the conclusion that the claims encompass an unlimited number of polypeptides and nucleic acids. Consequently, the Office argues that "the specification fails to disclose a representative number of species to describe the claimed genus," and cites *The Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997).

Applicants first point out that, for the reasons given above, the specification provides ample guidance on the requirements for a sialyl Le^x addition site and a tyrosine sulfation site. Therefore, the claims are not of unlimited scope, as the Office suggests.

Moreover, Applicants respectfully submit that the Office has misapplied *Lilly* to the instant case. The *Lilly* court, citing *In re Grimme*, 274 F.2d 949, 952, 124 U.S.P.Q. 499, 501 (Cust. & Pat. App. 1960), specifically noted that "it may not be necessary to enumerate a plurality of species if a genus is sufficiently identified in an application by other appropriate language." *Lilly* at 1569. As an alternative to setting forth large number of species falling within a claimed genus, the *Lilly* court explicitly provides for "a recitation of structural features common to the members of the genus, which features

constitute a substantial portion of the genus." *Id.* at 1569 (emphasis added). The present application does exactly that. In addition to providing a working embodiment, specific structural elements are identified by Applicants as characterizing an artificial P-selectin ligand. Both the tyrosine sulfation site and the sialyl Le^x addition site are classic motifs with highly defined structures which impart predictable properties to the protein. As described in detail above, both of these consensus sites are well recognized in the art and easily identified. In view of the fact that Applicants define their genus by the presence of specific structural features — exactly as required by the *Lilly* court — the written description requirement is satisfied, and this basis for the § 112 rejection may be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 10, 12-14, 24, and 25 are rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Specifically, the Office asserts that the terms "sialyl Le^x addition site" and "tyrosine sulfation site" are unclear and do not adequately describe the claimed invention. Applicants respectfully disagree.

Applicants note that the claims have been narrowed to recite only N-linked sialyl Le^x addition sites. As discussed above, sialyl Le^x addition is art-recognized to occur primarily at the consensus site N X S/T, which is disclosed at page 25, lines 19-27 of the specification. Therefore, the specification in combination with the prior art clearly define the metes and bounds of a sialyl Le^x addition site.

Likewise, as discussed above, it is well known that sulfation does not occur on any and every tyrosine. Flanking amino acid residues impart specific sequence characteristics necessary for tyrosine sulfation to occur. These consensus sequences are well recognized in the art, as also indicated above. Consequently, the term "tyrosine sulfation site" is a term of art used to encompass only those very specific tyrosine residues that are flanked by amino acid sequences having well defined properties.

The terms "sialyl Le^x addition site" and "tyrosine sulfation site" are not indefinite; rather, each has a specific, well-recognized definition and limitations. Applicants respectfully request this rejection be withdrawn.

CONCLUSION

Applicants submit that this case is now in condition for allowance, and such action is requested. Enclosed is a petition to extend the period for replying for three months, to and including September 21, 2001. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Claims as Pending

10. A purified nucleic acid encoding a polypeptide that is a synthetic P-selectin ligand, wherein said polypeptide contains an N-linked sialyl Le^x addition site and a tyrosine sulfation site, and wherein at least one of the sites is located at an amino acid position in said polypeptide which is different from its position in a naturally-occurring P-selectin ligand.

12. The purified nucleic acid of claim 10, wherein said nucleic acid further encodes an antibody or antibody fusion protein.

13. A vector comprising the nucleic acid of claim 10.

14. A cell comprising the nucleic acid of claim 10.

24. The purified nucleic acid of claim 10, wherein said tyrosine sulfation site consists of the Factor VIII tyrosine sulfation sequence set forth in SEQ ID NO: 15.

25. The purified nucleic acid of claim 10, wherein said polypeptide comprises Ile135 through Ser225 of the CD43 precursor sequence (SEQ ID NO: 17).

Version With Markings to Show Changes Made

10. A purified nucleic acid encoding a polypeptide that is a synthetic P-selectin ligand, wherein said polypeptide contains [a] an N-linked sialyl Le^x addition site and a tyrosine sulfation site, and wherein at least one of the sites is located at an amino acid position in said polypeptide which is different from its position in a naturally-occurring P-selectin ligand.